

What is claimed is:

1. A method for detecting a mutation in a nucleic acid molecule which comprises:
 - 5 (a) contacting the nucleic acid molecule with a probe comprising two covalently linked nucleic acid segments under conditions such that the unlinked end of each segment of the probe is capable of hybridizing with the nucleic acid molecule;
 - 10 (b) contacting the mixture from step (a) with a ligase under conditions such that the two hybridized probe segments will ligate and bind the nucleic acid molecule if the nucleic acid molecule contains the mutation;
 - 15 (c) determining the presence of bound nucleic acid molecule(s) and thereby detecting the mutation in the nucleic acid molecule.
- 20 2. The method of claim 1, wherein the nucleic acid molecule is a DNA molecule.
- 25 3. The method of claim 1, wherein the nucleic acid molecule is an RNA molecule.
4. The method of claim 1, wherein the nucleic acid molecule is a mitochondrial DNA molecule.
- 30 5. The method of claim 1, wherein the nucleic acid molecule is a circular DNA molecule.
- 35 6. The method of claim 1, wherein the nucleic acid molecule is a chromosomal DNA molecule.

7. The method of claim 1, wherein the nucleic acid molecule is a viral DNA molecule.

5 8. The method of claim 1, wherein the nucleic acid molecule is a cDNA molecule.

9. The method of claim 1, wherein the nucleic acid molecule is greater than 800 bases long.

10 10. The method of claim 1, wherein the nucleic acid molecule is greater than 2 kilobases long.

15 11. The method of claim 1, wherein the probe segments comprise nucleotides modified in their sugar, phosphate or base.

20 12. The method of claim 11, wherein the modified nucleotide is a phosphorothioate, phosphoramidate, phosphorodithioate, peptide nucleic acid, phosphonate, methylphosphonate or phosphate ester.

13. The method of claim 1, wherein the two probe segments are covalently linked by an oligonucleotide.

25 14. The method of claim 1, wherein the probe is labeled with a detectable moiety.

30 15. The method of claim 14, wherein the detectable moiety is a fluorescent label, a radioactive atom, a chemiluminescent label, a paramagnetic ion, biotin or a label which can be detected through a secondary enzymatic or binding step.

35 16. The method of claim 1, wherein the determination of the presence of bound nucleic acid molecule(s) is by an

enzymatic reaction.

17. The method of claim 1, wherein the determination of the presence of bound nucleic acid molecule(s) is by fluorescence.

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18. The method of claim 1, wherein the determination of the presence of bound nucleic acid molecule(s) is by chemiluminescence.

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19. The method of claim 1, wherein the determination of the presence of bound nucleic acid molecule(s) is by magnetic charge.

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20. The method of claim 1, wherein the probe is attached to an affinity medium.

21. The method of claim 1, wherein the nucleic acid molecules are attached to the affinity medium.

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22. The method of claim 1, wherein the binding of the nucleic acid molecule comprises catenation.

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23. The method of claim 1, wherein the mutation(s) is a point mutation.

24. The method of claim 1, wherein the mutation(s) is a deletion mutation.

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25. The method of claim 1, wherein the mutation(s) is an insertion mutation.

26. The method of claim 1, wherein the mutation(s) is a translocation mutation.

27. The method of claim 1, wherein the mutation(s) is an inversion mutation.

28. The method of claim 1, wherein the nucleic acid molecule contains a plurality of detectable mutations.

5 29. A method for diagnosing a genetic disorder in a subject which comprises:

10 (a) contacting a sample of bodily fluid or tissue from the subject containing a nucleic acid molecule(s) which may be associated with the genetic disorder, with a probe comprising two covalently linked nucleic acid segments under conditions such that the unlinked end of each segment of the probe is capable of hybridizing with the nucleic acid molecule(s);

15 (b) contacting the mixture from step (a) with a ligase under conditions such that the two hybridized probe segments ligate and bind the nucleic acid molecule(s) if the nucleic acid molecule(s) is associated with the genetic disorder;

20 (c) determining the presence of any bound nucleic acid molecule(s), detecting the nucleic acid molecule(s) and thereby diagnosing the genetic disorder.

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30 30. The method of claim 29, wherein the nucleic acid molecule(s) is covalently linked to a solid support.

35 31. The method of claim 29, wherein the probe(s) is covalently linked to a solid support.

32. The method of claim 29, wherein the solid support is a microscope slide comprised of plastic or glass, either uncoated or coated with a suitable attachment substrate.

5 33. The method of claim 29, wherein the solid support is a nylon membrane, a cellulose acetate membrane, an epoxy-activated synthetic copolymer membrane or a nitrocellulose membrane.

10 34. The method of claim 29, wherein the solid support is a tube or bead or any part thereof, which is sepharose, latex, glass or plastic.

15 35. The method of claim 29, wherein the probe is labeled with a detectable moiety.

20 36. The method of claim 35, wherein the detectable moiety is a flourescent label, a radioactive atom, a chemiluminescent label, a paramagnetic ion, biotin or a label which can be detected through a secondary enzymatic or binding step.

25 37. The method of claim 29, wherein the determination of the presence of bound nucleic acid molecule(s) is by an enzymatic reaction.

30 38. The method of claim 29, wherein the determination of the presence of bound nucleic acid molecule(s) is by fluorescence.

35 39. The method of claim 29, wherein the determination of the presence of bound nucleic acid molecule(s) is by chemiluminescence.

40. The method of claim 29, wherein the determination of the presence of bound nucleic acid molecule(s) is by magnetic charge.

5 41. The method of claim 29, wherein the nucleic acid molecules are attached to an affinity medium.

42. The method of claim 29, wherein the binding comprises catenation.

10 43. The method of claim 29, wherein the genetic disorder is associated with a point mutation.

15 44. The method of claim 29, wherein the genetic disorder is associated with a deletion mutation.

45. The method of claim 29, wherein the genetic disorder is associated with an insertion mutation.

20 46. The method of claim 29, wherein the genetic disorder is associated with a translocation mutation.

47. The method of claim 29, wherein the genetic disorder is associated with an inversion mutation.

25 48. The method of claim 29, wherein the nucleic acid molecule contains a plurality of detectable genetic disorders.

30 49. A method for identifying neutral polymorphisms in a subject which comprises:

35 (a) contacting a sample of bodily fluid or tissue from the subject containing a nucleic acid molecule(s) which may be associated with the

neutral polymorphism, with a probe comprising two covalently linked nucleic acid segments under conditions such that the unlinked end of each segment of the probe is capable of hybridizing with the nucleic acid molecule(s);

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(b) contacting the mixture from step (a) with a ligase under conditions such that the two hybridized probe segments ligate and bind the nucleic acid molecule(s) if the nucleic acid molecule(s) is associated with the neutral polymorphism;

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(c) determining the presence of any bound nucleic acid molecule(s), detecting the nucleic acid molecule(s) and thereby identifying the neutral polymorphism(s).

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50. A method for selecting a particular mutation in a nucleic acid molecule from a population of engineered nucleic acid molecules containing random mutations, which comprises:

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(a) contacting a sample containing the nucleic acid molecule(s) which may contain the particular mutation, with a probe comprising two covalently linked nucleic acid segments under conditions such that the unlinked end of each segment of the probe is capable of hybridizing with the nucleic acid molecule(s);

(b) contacting the mixture from step (a) with a ligase under conditions such that the two hybridized probe segments ligate and bind the nucleic acid molecule(s) if the nucleic acid

molecule(s) contain the particular mutation;

(c) selecting any bound nucleic acid molecule(s), and thereby selecting the nucleic acid molecule(s) containing the particular mutation(s) from the population.

5 51. The method of claim 50, wherein the nucleic acid molecule(s) is covalently linked to a solid support.

10 52. The method of claim 50, wherein the probe(s) is covalently linked to a solid support.

15 53. The method of claim 50, wherein the solid support is a microscope slide comprised of plastic or glass. material

54. The method of claim 50, wherein the solid support is a nylon or nitrocellulose membrane.

20 55. The method of claim 50, wherein the solid support is a bead which is sepharose, latex, glass or plastic.

56. The method of claim 50, wherein the probe is labeled with a detectable moiety.

25 57. The method of claim 56, wherein the detectable moiety is a fluorescent label, a radioactive atom, a chemiluminescent label, a paramagnetic ion, biotin or a label which can be detected through a secondary enzymatic or binding step.

30 58. The method of claim 50, wherein the selection of the bound nucleic acid molecule(s) is by an enzymatic reaction.

59. The method of claim 50, wherein the selection of the bound nucleic acid molecule(s) is by fluorescence.

60. The method of claim 50, wherein the selection of the bound nucleic acid molecule(s) is by chemiluminescence.

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61. The method of claim 50, wherein the selection of the bound nucleic acid molecule(s) is by magnetic charge.

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62. The method of claim 50, wherein the nucleic acid molecules are attached to an affinity medium.

63. The method of claim 50, wherein the binding comprises catenation.

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64. The method of claim 50, wherein the particular mutation is associated with a point mutation.

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65. The method of claim 50, wherein the particular mutation is associated with a deletion mutation.

66. The method of claim 50, wherein the particular mutation is associated with an insertion mutation.

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67. The method of claim 50, wherein the particular mutation is associated with an inversion mutation.

68. A kit for detecting the presence of one or more mutation(s) in a nucleic acid molecule which comprises:

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(a) a solid support having a plurality of covalently linked probes which may be the same or different, each probe of which comprises two covalently linked nucleic acid segments which are capable of

being ligated and detected in the presence of the nucleic acid molecule, if the nucleic acid molecule contains one or more mutation(s).

5 69. The kit of claim 68 further comprising:

10 (b) a means for ligating each probe segment pair such that the two hybridized probe segments ligate intramolecularly and bind the nucleic acid molecule if the nucleic acid molecule contains one or more mutation(s).

70. The kit of claim 69 further comprising:

15 (c) a means for determining the presence of bound nucleic acid molecule(s) so as to detect the presence of mutation(s) in the nucleic acid molecule.

20 71. The kit of claim 68, wherein the nucleic acid molecule is a DNA molecule.

72. The kit of claim 68, wherein the nucleic acid molecule is an RNA molecule.

25 73. The kit of claim 68, wherein the nucleic acid molecule is a mitochondrial DNA molecule.

74. The kit of claim 68, wherein the nucleic acid molecule is a circular DNA molecule.

75. The kit of claim 68, wherein the nucleic acid molecule is a chromosomal DNA molecule.

30 76. The kit of claim 68, wherein the nucleic acid molecule

is a viral DNA molecule.

77. The kit of claim 68, wherein the nucleic acid molecule is a cDNA molecule.

5 78. The kit of claim 68, wherein the nucleic acid molecule is greater than 800 bases long.

10 79. The kit of claim 68, wherein the nucleic acid molecule is greater than 2 kilobases long.

80. The kit of claim 68, wherein the nucleic acid segments of the plurality of probes are not identical.

15 81. The kit of claim 68, wherein the probe segments comprise nucleotides modified in their sugar, phosphate or base.

20 82. The kit of claim 81, wherein the modified nucleotide is a phosphorothioate, phosphoramidate, phosphorodithioate, peptide nucleic acid, phosphonate, methylphosphonate or phosphate ester.

25 83. The kit of claim 68, wherein the two probe segments are covalently linked by an oligonucleotide.

84. The kit of claim 68, wherein the probe is labeled with a detectable moiety which is a fluorescent label, a radioactive atom, a chemiluminescent label, a paramagnetic ion, biotin or a label which can be detected through a secondary enzymatic or binding step.

30 85. The kit of claim 68, wherein the probe is designed to detect at least one point mutation.

86. The kit of claim 68, wherein the probe is designed to detect at least one deletion mutation.
87. The kit of claim 68, wherein the probe is designed to detect at least one insertion mutation.
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88. The kit of claim 68, wherein the probe is designed to detect at least one inversion mutation.
89. The kit of claim 68, wherein the probe is designed to detect a plurality of mutations.
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